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European Journal of Pharmacology 442 (2002) 225–229

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Short communication

In vivo tonic modulation of the noradrenaline release in the rat cortex by locus coeruleus somatodendritic α_2-adrenoceptors

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Received 3 January 2002; received in revised form 13 March 2002; accepted 19 March 2002

Abstract

The regulation of noradrenaline release in the rat cingulate cortex by somatodendritic α_2-adrenoceptors placed in the locus coeruleus was evaluated by dual-probe microdialysis. The α_2-adrenoceptor antagonists BRL44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole), RS79948 ((8,12,13)-decahydro-3methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine) and RX821002 (2-methoxyidazoxan) administered by reverse dialysis into the locus coeruleus increased concentration-dependently (0.01–100 μM) noradrenaline release in the cortex (maximal effects 170 ± 30%, 543 ± 17%, 195 ± 26%, respectively). Administration of the α_2-adrenoceptor antagonist idazoxan increased at lower (0.1–10 μM) but decreased at the highest dose (100 μM) noradrenaline in the cortex. These data demonstrate that somatodendritic α_2-adrenoceptors in the locus coeruleus exert an inhibitory tonic modulation on noradrenaline release in noradrenergic terminal areas.

Keywords: α_2-Adrenoceptor; Noradrenaline; Locus coeruleus; Cortex; Cingulate; Microdialysis; Somatodendritic release

1. Introduction

The locus coeruleus is the most important source of noradrenergic innervation in the central nervous system. It is well established that the firing activity of locus coeruleus neurones is controlled, among others, by somatodendritic α_2-adrenoceptors placed in the area. Thus, the systemic or microiontophoretic administration of the α_2-adrenoceptor agonist clonidine inhibits the firing activity of locus coeruleus noradrenergic cells (Svensson et al., 1975). Accordingly, infusion of clonidine into the locus coeruleus promotes a decrease of the extracellular concentration of noradrenaline in the ipsilateral cortex (Van Gaalen et al., 1997; Mateo and Meana, 1999). In the locus coeruleus, noradrenaline release (Van Gaalen et al., 1997; Mateo et al., 1998) and uptake (Thomas et al., 1994; Mateo et al., 1998) processes have been demonstrated in vivo. Therefore, a tonic autoinhibitory regulation of locus coeruleus noradrenergic neurones by local noradrenaline acting on somatodendritic α_2-adrenoceptors has been proposed. However, the existence of this tonic inhibitory modulation is controversial. Thus, some studies have shown increased firing rate of locus coeruleus neurones or enhanced noradrenaline release in terminal areas induced by local administration of α_2-adrenoceptor antagonists into the locus coeruleus (Cedarbaum and Aghajanian, 1976; Mateo and Meana, 1999; Pudovkina et al., 2001). In contrast, other electrophysiological (Freedman and Aghajanian, 1984) and microdialysis studies (Kawahara et al., 1999; Linnér et al., 1999) indicate that systemic or local perfusion of the α_2-adrenoceptor antagonist idazoxan does not induce changes in locus coeruleus activity. This fact suggests the lack of tonic modulation of locus coeruleus neurones by α_2-adrenoceptors in vivo. However, idazoxan also displays important affinity for other receptors expressed in the locus coeruleus area (Hoyer, 1988; Miralles et al., 1993; Newman-Tancredi et al., 1998).

The present study sought to investigate the effect of different α_2-adrenoceptor antagonists on the noradrenaline release in the cingulate cortex when the drugs are locally applied into the locus coeruleus by reverse dialysis (Van Gaalen et al., 1997; Mateo and Meana, 1999). The influence of somatodendritic α_2-adrenoceptors on the tonic regulation of locus coeruleus activity was evaluated by measuring extracellular noradrenaline in the cingulate cortex, a projection area arising from the locus coeruleus. Actions of various α_2-adrenoceptor antagonists were compared with those of idazoxan.

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01543-1

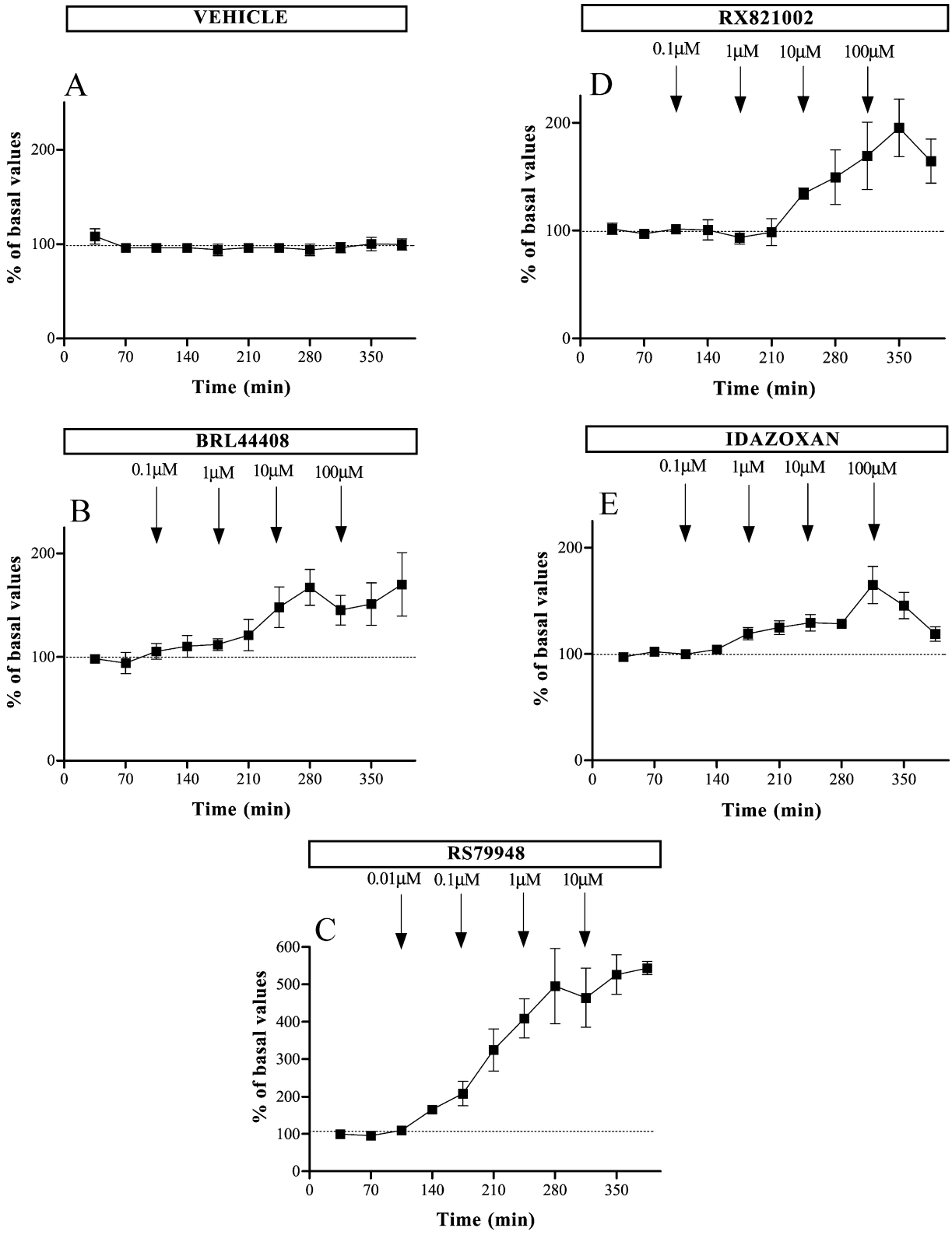

Fig. 1. Effects of local administration into the locus coeruleus area of artificial CSF (A) and the α_2-adrenoceptor antagonists BRL44408 (B), RS79948 (C), RX821002 (D) or idazoxan (E) on extracellular noradrenaline concentrations measured in the rat cingulate cortex by in vivo microdialysis. Drugs were dissolved in the artificial CSF and administered by reverse dialysis in increased concentrations every two fractions (70 min) in 10-fold increments (arrows). Data are mean ± S.E.M. (bars) values from four to five separate experiments and are expressed as percentages of the corresponding baseline values.

2. Materials and methods

2.1. Animals, surgery and microdialysis procedures

Male Sprague–Dawley rats (250–300 g) were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and placed for stereotaxic surgery as previously reported (Mateo et al., 1998). One microdialysis probe (2.0 × 0.25 mm) was implanted in the vicinity of the right locus coeruleus (AP − 3.7; L + 1.3; V − 8.2, taken in millimeters from lambda suture point) and the other probe (4.0 × 0.25 mm) was implanted in the ipsilateral cingulate cortex (AP + 2.8; L + 1; V − 5.0, taken in millimeters from bregma).

Microdialysis experiments were carried out in freely moving animals around 20 h after probe implantation. The probes were perfused with a modified cerebrospinal fluid (CSF) solution (148 mM MgCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$, pH 7.4) at a flow rate of 1 μl/min. Dialysates were collected every 35 min and following 1 h for stabilisation, three samples were collected before drug infusions. Drugs were dissolved in the artificial CSF and perfused by reverse dialysis through the probe place into the locus coeruleus area in increasing concentrations (0.01–100 μM). Each concentration was perfused for two sampling periods (70 min). This period has been shown suitable to produce a steady-state effect of a given dose of clonidine on extracellular noradrenaline (Mateo and Meana, 1999). Control group was maintained under continuous perfusion of the artificial CSF solution (vehicle for drugs). After the experiments, the animals were killed and the brains dissected out to verify the correct implantation of the probes.

2.2. Chromatographic analysis

Immediately after collection, noradrenaline from dialysate samples was quantified by high-performance liquid chromatography (HPLC) with electrochemical detection, as previously described (Mateo et al., 1998).

2.3. Statistical analysis

The mean values of the three basal samples before drug administration were considered as the 100% basal value. Extracellular noradrenaline values of dialysate samples were normalised as a percentage of the corresponding basal values. The concentration–response effects of the drugs were assessed by one-way analysis of variance (ANOVA). All results are expressed as mean ± S.E.M. values and the level of the significance was chosen as $P = 0.05$.

2.4. Drugs

BRL44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole)-methyl]-4,5-dihydroimidazole) maleate and RS79948 ((8,12,13)-decahydro-3metoxy-12(ethylsulphonyl)-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine) HCl were supplied by Tocris Cookson (Bristol, UK). RX821002 (2-methoxyidazoxan) and (±)idazoxan HCl were synthesised at Lasa Laboratorios (Barcelona, Spain).

3. Results

Basal extracellular concentrations of noradrenaline were 3.84 ± 0.29 nM ($n = 19$). Maintained local administration into the locus coeruleus of artificial CSF (control group) did not change noradrenaline in the cingulate cortex ($F[10,30] = 0.53$, $P = 0.84$) (Fig. 1A).

The administration into the locus coeruleus of BRL44408 (0.1–100 μM) induced a concentration-dependent increase of noradrenaline in the cortex ($F[10,47] = 3.21$, $P < 0.01$), with a maximal effect of 170 ± 30%, reached at 100 μM (Fig. 1B). When the antagonist RS79948 was perfused into the locus coeruleus area (0.01–10 μM), a concentration-dependent enhancement of noradrenaline in the cortex was also observed ($F[10,39] = 11.43$; $P < 0.0001$). The maximal effect (543 ± 17%) was reached at 10 μM RS79948 (Fig. 1C). Further administration of RS79948 at 100 μM did not modify maximal noradrenaline concentration values obtained at 10 μM (data not shown).

In the same way, extracellular noradrenaline in the cortex was increased ($F[10,34] = 4.97$; $P < 0.0001$) by the perfusion into the locus coeruleus of RX821002 (0.1–100 μM) with a maximal effect of 195 ± 26% reached at 100 μM (Fig. 1D).

Finally, when idazoxan was locally perfused into the locus coeruleus, the concentration of noradrenaline in the cingulate cortex increased concentration-dependently in the range 0.1 to 10 μM ($F[8,39] = 4.21$, $P < 0.0001$) with a maximal effect of 164 ± 17% obtained at 10 μM idazoxan. However, at higher concentrations, idazoxan perfusion (100 μM) reversed to control values (maximal effect 118 ± 16%) noradrenaline concentration in the cingulate cortex with a statistically significant effect obtained 70 min after the start of the infusion (10 vs. 100 μM values, $P < 0.01$, Bonferroni's multiple comparison test) (Fig. 1E).

4. Discussion

The present results support and confirm that somatodendritic α_2-adrenoceptors placed in the locus coeruleus area exert a tonic autoinhibitory in vivo regulation on the noradrenaline release in the cingulate cortex.

Previous studies have shown an inhibitory effect on the release of noradrenaline mediated by the presynaptic α_2-adrenoceptors placed as terminal autoreceptors. Thus, local administration of α_2-adrenoceptor agonists decreases extracellular noradrenaline in brain cortex (Van Veldhuizen et al., 1993; Dalley and Stanford, 1995). Conversely, the local administration of different α_2-adrenoceptor antagonists in noradrenergic terminal areas increases noradrenaline (Dennis et al., 1987; Thomas and Holman, 1991; Van Veldhuizen

et al., 1993; Dalley and Stanford, 1995; Wortley et al., 1999a), indicating a tonic action on these autoreceptors by the endogenous neurotransmitter. However, the similar effects of local (terminal areas) and systemic administration of α_2-adrenoceptor drugs have led to indirectly suggest the absence of a tonic inhibitory activity mediated by somatodendritic α_2-adrenoceptors in vivo (Dennis et al., 1987; Van Veldhuizen et al., 1993). In the present study, the effect of α_2-adrenoceptor antagonists on cortical noradrenaline was evaluated by direct local administration into the locus coeruleus. The study was performed in a wide range of drug concentrations and for several α_2-adrenoceptor antagonists with different pharmacological profiles. The results agree with previous studies in which local administration of α_2-adrenoceptor antagonists induced excitation of locus coeruleus neurones (Cedarbaum and Aghajanian, 1976) and increased noradrenaline release in cortical areas (Mateo and Meana, 1999; Pudovkina et al., 2001).

The compound BRL44408 is considered a non-high-potency drug but the most selective antagonist of the α_{2A}-adrenoceptor subtype (Renouard et al., 1994). The observations herein are compatible with this pharmacological profile. Furthermore, the present finding is a new evidence that in vivo noradrenaline release in the cingulate cortex is modulated by somatodendritic α_2-adrenoceptors that correspond to the α_{2A}-adrenoceptor subtype (Nörenberg et al., 1997; Mateo and Meana, 1999; Pudovkina et al., 2001).

Idazoxan, when locally applied into the locus coeruleus area, increased noradrenaline in the cortex at low concentrations but reversed the noradrenaline increase at the highest concentrations. In this context, other authors have observed no change of noradrenaline release in rat prefrontal cortex following local administration of a single concentration of idazoxan (50 μM) into the locus coeruleus area (Kawahara et al., 1999). The finding was interpreted as an evidence that somatodendritic α_2-adrenoceptors do not play an autoinhibitory role on locus coeruleus neuronal activity. Idazoxan is a potent α_2-adrenoceptor antagonist drug that displays important affinity for imidazoline receptors (Miralles et al., 1993) and 5-HT_{1A} receptors (Hoyer, 1988; Lladó et al., 1996; Newman-Tancredi et al., 1998), which are expressed in the locus coeruleus area. Taking this into account, to establish conclusions about the control of noradrenaline release in the cingulate cortex on the basis of a single idazoxan effect in the locus coeruleus might be speculative. Nevertheless, the present results clearly show that under suitable concentrations of idazoxan, tonic autoinhibitory modulation of cortical noradrenaline release can be also achieved.

The α_2-adrenoceptor antagonist RX821002 (2-methoxyidazoxan) also displays moderate affinity for 5-HT_{1A} receptors (Meana et al., 1996; Ogilvie and Clarke, 1998). Although different responses of extracellular noradrenaline to systemic RX821002 have been reported (Mateo et al., 1998; Wortley et al., 1999a,b), no previous data are available based on local administration into the locus coeruleus. In fact, the attenuation of noradrenaline increase at the highest concentration of RX821002 (see Fig. 1D) could represent the effect of this compound on 5-HT_{1A} receptors.

Affinity for 5-HT_{1A} receptors is a common feature of many other α_2-adrenoceptor antagonists (De Vos et al., 1991; Meana et al., 1996; Newman-Tancredi et al., 1998). The compound RS79948 represents a very potent α_2-adrenoceptor antagonist (Brown et al., 1993) that displays a great selectivity for α_2-adrenoceptors over 5-HT_{1A} receptors (Brown et al., 1993). In agreement with these previous data, the local administration of RS79948 into the locus coeruleus area markedly enhanced the release of noradrenaline in the cingulate cortex. This larger increase of cortical noradrenaline induced by RS79948 might be explained by the important affinity of the compound for α_2-adrenoceptors and the dissimilar pharmacological profile with other α_2-adrenoceptor antagonists. An alternative explanation include a putative inverse agonist activity of RS79948. Although some in vitro functional studies have discarded such a possibility (Brown et al., 1993), the precise pharmacological identity of RS79948 is still unclear.

In conclusion, the present work demonstrates that somatodendritic α_2-adrenoceptors located in the locus coeruleus exert a tonic autoinhibitory effect in vivo on the noradrenaline release in the cingulate cortex. In particular, the pharmacological profile of idazoxan should be born in mind when conclusions on the functional role of α_2-adrenoceptors will be drawn. RS79948 appears as a suitable alternative tool for the study of α_2-adrenoceptors.

Acknowledgements

This work was supported by grants from FIS (98/0559), UPV/EHU (GI 13/98) and MCYT (SAF 2001-0553). B.F.-P. is supported by a predoctoral fellowship from the Basque Government.

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